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CHEMICAL CONTROL OF STEM END ROT OF MANGO CAUSED BY *LASIODIPLODIA THEOBROMAE*

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A B S T R A C T

Stem end rot is the most severe and widely prevailed postharvest disease of mango throughout the Pakistan. *Lasiodiplodia theobromae* was predominantly isolated from the mango fruits having typical symptoms of the stem end rot disease. Pathogenicity assay depict that faster and severe symptoms appear on mango fruit when they inoculated with disks of *L. theobromae* as compared to injection method. Range of chemical fungicides is use in pre- and post-harvest disease management. In present comparative studies of six fungicides [Carbendazim (Carbendazim), Gemstar (Azoxystrobin), Native (Tebuconazole+Trifloxystobin), Score (Difenoconazole), Tecto (Thiabendazole) and Tilt (Propiconazole)], lower used concentrations of Carbendazim followed by Tecto appeared as highly effective to inhibit the growth of *L. theobromae* on agar medium while at higher used concentration (30000 ppm) no growth of *L. theobromae* was observed with any fungicide. Generally, higher concentration of fungicides were more effective than the lower concentrations All fungicides more or less checked the pathogen infection on mango fruits inoculated with *L. theobromae*, as significantly minimum disease development was observed on treated fruits as compared to the untreated once (control). Nativo, Gemstar and Carbedazim at the lowest used dose (10000 ppm) reduced the lesion area while no lesion develops at 20000 and 30000 ppm in hot dip treatment at 50 ºC for 5 min.

Keywords: *Mangifera indica*, Postharvest rot, Sindhri, Fungicides, Postharvest treatment.

INTRODUCTION

Domestic and international trade of fresh mango has been limited by its highly perishable nature and its susceptibility to postharvest diseases. Postharvest diseases of mango reduce fruit quality and cause severe losses, because they leave them completely unmarketable (Bally *et al.,* 2009; Barkai-Golan, 2001; Narayanasam, 2006). Postharvest losses of fresh mango fruits are reported to be 25-40% in India and 69% in Pakistan, and microbial decay accounts for 17.0-26.9% of the total postharvest losses in Asian countries (Prabakar *et al.,* 2005). Postharvest losses may be due to various factors, including fungal pathogens which play a major role in postharvest rotting of mangoes. Major postharvest diseases that deteriorate the fruit quality include anthracnose, stem end rot and soft nose (Cappellini *et al.,* 1988; Jeffries *et al*., 1990; Crane and Campbell, 1991). Sharma *et al*. (1994) reported that 17 pathogens associated with postharvest diseases in Himachal Pradesh, India, in 1990-92. Our recent studies on "Mango Postharvest Disease Assessment of Sindh, Pakistan Orchards" revealed the prevalence of number of fungi including *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus nigar*, *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Phomopsis mangiferae* and *Rhizopus stolonifier*.

The cv. Sindhri is most popular and potential variety found to highly susceptible to the stem end rot disease. This disease is caused by a complex of fungal pathogens, of which various *Botryosphaeria* spp. are dominant (Darvas, 1991; Johnson *et al.,* 1991, Sangchote, 1991). *Botryodiplodia theobromae* (Pat.) Griffon & Maubl., is geographically widespread specie of Botryosphaeriaceae to tropics and subtropics region (Punithalingam, 1980; Johnson *et al*., 1992). It was responsible for 26.7% of decay diseases in Himachal Pradesh, India, in 1990-92 (Sharma *et al*., 1994). A number of postharvest technologies are developed to reduce postharvest losses, including pre-harvest management, proper and timely

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harvesting and transport to the packhouse. Packhouse measures, pre- and post-shipping storage, transport and marketing (Johnson and Hofman, 2009). Chemicals are most widely used, to control the postharvest diseases of mango. The most widely used fungicide such as Benomyl banned from different countries of the world due to chemical residues issue. Moreover, the postharvest hot water treatment was also found ineffective against mango stem end rot disease (Coates *et al*., 1993). Emphasis must be given to exploring reduced risk chemicals as well as other non-chemical methods to control the postharvest quality of mango. Therefore, present studies are conducted to find out most suitable chemical control of mango stem end rot caused by *L. theobromae* on most popular mango variety 'Sindhri'.

MATERIALS AND METHODS

Pathogenicity of *L. theobromae:* *L. theobromae* was isolated from the infected mangoes showing stem end rot symptoms. Pathogenic nature of the isolated fungus was determined by inoculating the un-ripend mature harvested mangoes by four different methods of inoculation. Prior to inoculation, fruits were surface sterilized with 5% sodium hypochloride solution. The culture disks (5mm) of *L. theobromae* were cut from the actively growing culture on PDA medium and three were placed on one side of fruits (1st method) or injecting the 0.02 milliliter inoculum suspension containing $1x10^6$ conidia per milliliter (Awa *et al*., 2012) on the stem end (2nd method), three disks were placed on three different side near stem end (3rd method) or placed on the stem end after trimming the stem (4th method). Before placing the disk the skin was injured with a needle. After inoculation, fruits were placed inside the bell jar (Fig. 1), lined from bottom with moistened sterilized blotting paper to avoid desiccation and incubated at room temperature. After 12 hour of incubation culture disks were removed and fruits were transferred to air conditioned room (20 ºC) for symptoms development.

Figure 1. Incubation of the inoculated fruits in bell jar, lined from bottom with moistened blotting paper to avoid desiccation.

***In-vitro* screening of fungicides:** Six different fungicide namely Carbendazim, Gemstar, Nativo, Score, Tecto, Tilt, were evaluated with seven different concentration (1, 10, 100, 1000, 10000, 20000 and 30000 ppm) by food poisoning method (Borum and Sinclair, 1968) against *L. theobromae*. The detail of fungicides including their brand name, active ingredient, concentration/formulation and manufacture are given in Table 1.

Table 1. Details of the fungicides used in the present studies.

Brand Name	Active ingredient	Concentration/Formulation	Manufacture
Carbendazim	Carbendazim	50WP	Agri Aid Enterprise
Gemstar	Azoxystrobin	250EC	Suncrop Pesticides
Nativo	(Tebuconazole+ Trifloxystobin)	75WG	Bayer
Score	Difenoconazole	250EC	Syngenta
Tecto	Thiabendazole (Benzimidazole)	500SC	Syngenta
Tilt	Propiconazole	250EC	Syngenta

The required concentrations of the fungicides were added in the PDA medium before pouring, concentration were calculated on the basis of active ingredient of fungicide and maintained by serial dilution method. Medium without fungicide served as control. After solidifying of the medium, 5mm diameter agar disk of test fungus were cut from 8-10 days old culture plate by using sterile cork borer and placed in the center of the PDA plate. The inoculated plates were incubated at 25ºC. The radial colony growth of test fungus were recorded by drawing two perpendicular lines on the back of the Petri plates crossed each other in the center of the plate. The data on colony growth was recorded along with these lines in millimeter after each 24 hours until the plates were filled in any treatment.

Evaluation of different fungicides on disease development: Above mention fungicide was also used to see their effect on disease development. Mangoes were inoculated as described in pathogenicity test by placing three disks on one side of the mango. Fruits were kept in moist chamber and after 12 hours of inoculation disk of the inoculum were removed. Fungicide suspension (10000, 20000, 30000 ppm) were prepared in hot water bath at 50 ºC. Mangoes were dipped for 5 min in aqueous solution of fungicide with continuous agitation of suspension, then dried by placing on blotting paper and transferred to air conditioned room (20 ºC) with relative humidity of 80 to 85 percent for symptoms development. Developed lesions area were recorded with the help of ImageJ software (http://imagej.nih.gov/ij/).

RESULTS

Pathogenicity of *L.* theobromae: Symptoms of stem end rot include as described by Johnson *et al* (2012) "Fast moving dark lesions are produced by *L. theobromae.* Fingers of watery decay begin to appear around the stem end, colonization extending ahead of symptoms along vascular tissue, colonization extending into the fruit flesh and the seed coat" *L. theobromae* was isolated from the infected mangoes showing stem end rot. The development of stem end rot disease on inoculated mango fruits greatly dependent on the method of pathogen inoculation (Fig. 2). The data depict that typical symptoms appear on mango fruits, intensities varies with the inoculation method of *L. theobromae*. The significantly ($p<0.05$) maximum lesion size was developed on fruits where inoculum disks of *L. theobromae* were placed on the side of the fruits (method 1) followed by 3rd and 4th inoculation method. The injection method (2nd method) appeared least effective, as it produced significantly minimum disease on inoculated fruits (Fig 2 & 3).

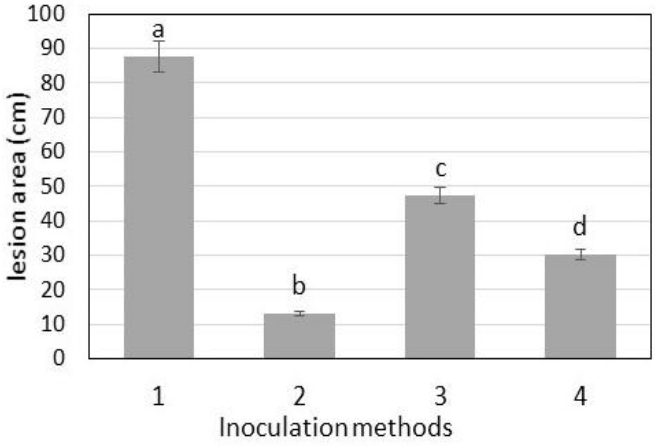

Figure 2. Effect of different inoculation methods on stem end rot disease development (area covered by the disease lesion) on mango fruits. Bar with different letters show significant difference ($P\leq0.05$) as determined by Duncan's Multiple Range Test.

Where;

Inoculation method 1: Three 5mm disks were placed on one side of fruits.

Inoculation method 2: Injecting the inoculum suspension of *L. theobromae* on the stem end. Inoculation method 3: Three 5mm disks were placed on three different sides near stem end.

Inoculation method 4: One 5mm disks of *L. theobromae* was placed on the stem end after trimming the stem.

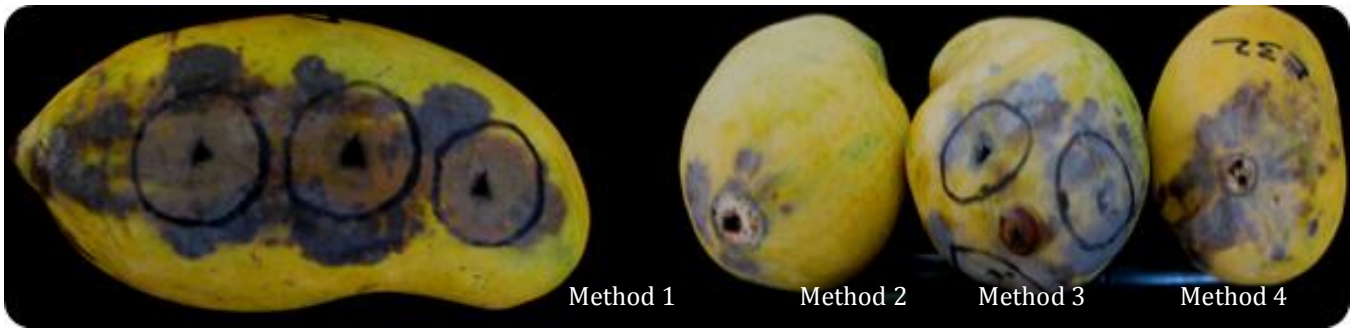

Figure 3. Influence of different inoculation method on the disease development on mango fruits (cv. Sindhri) inoculated with *L. theobromae*

Effect of fungicides on colony growth of *L. theobromae*: Among six fungicides, Carbendazim and Tecto appeared as highly effective to inhibit the growth of *L. theobromae* followed by other fungicides. The growth response of *L. theobromae* to different concentration of fungicides used varied greatly within the different treatments (Fig. 4). Generally, higher concentrations of fungicides were more effective than

the lower concentrations. In most cases, a gradual decline in pathogen growth noted with an increasing concentration of fungicides, except in case of Carbendazim and Tecto. In Carbendazim, pathogen can able to grow only at 1ppm, while its growth was completely checked at all other concentration. Similarly, in case of Tecto, *L. theobromae* could grow at 1 and 10 ppm, while at 100-20000 ppm it failed to grow. Gemstar and Tilt was also moderately effective against *L. theobromae*, the fungus failed to grow at 20000-30000 ppm of Gemstar and very little growth produce at 10000 ppm. A similar trend also observed in case of Tilt (Fig. 4).

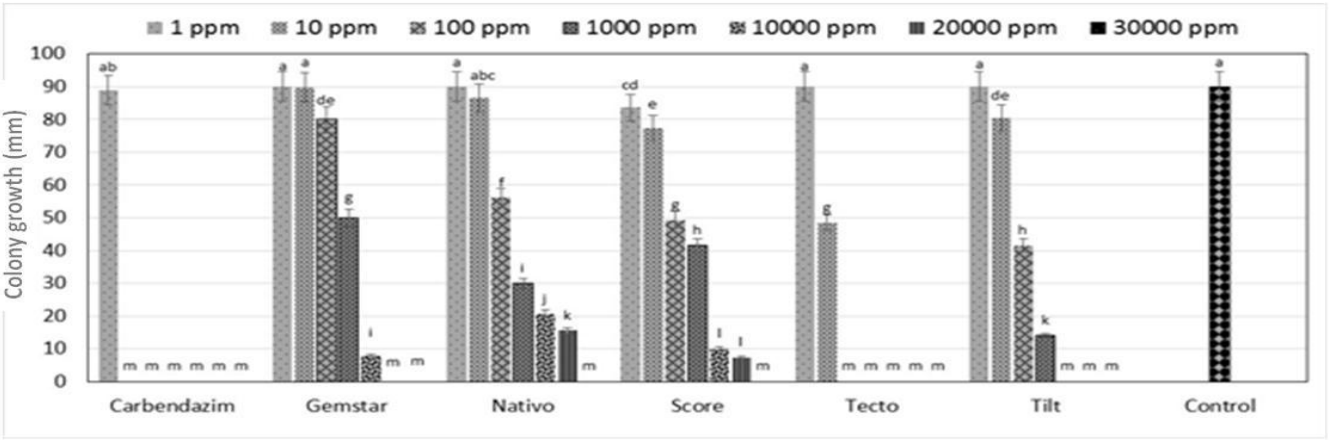

Figure 4. Effect of different concentrations of various fungicides on the colony growth of the *L. theobromae.* Bar with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

Effect of fungicides on disease development: All fungicides were more or less checked the pathogen infection on mango fruits inoculated with *L. theobromae*, as significantly minimum disease development was observed on treated fruits as compared to the untreated once (control). Among six fungicides Nativo, Gemstar and Carbendazim were more effective than Score, Tecto and Tilt (Fig. 5). No disease symptoms (lesions) were appeared on mango fruits treated with 20000 and 30000 ppm of Carbendazim, Gemstar and Score. However, 10000 ppm of these fungicides unable to completely check the disease development and moderate size lesions (16.7-22.9 cm) were developed on treated fruits. In case of Tilt, Tecto and Score the disease severity was greatly reduced with increasing dose of these fungicides, however all three doses of Score, Tecto and Tilt did not completely checked the disease development on fruits (Fig. 5).

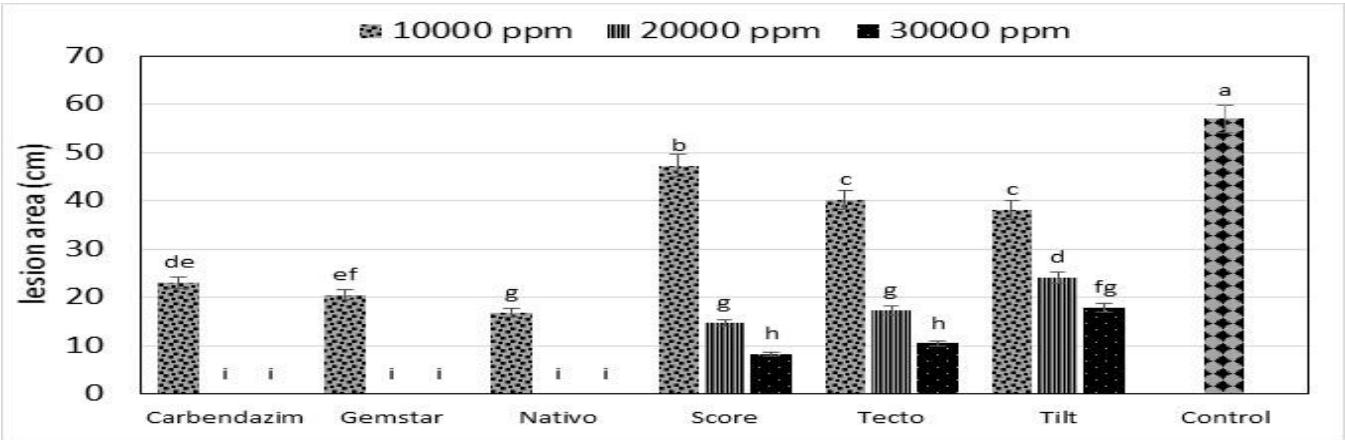

Figure 5. Effect of different concentrations of various fungicides on the stem end rot disease development on the mango fruits inoculated with *L. theobromae*. Bar with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

DISCUSSION

L. theobromae (Pat.) Griffon & Maubl., is geographically widespread specie of Botryosphaeriaceae to tropics and subtropics region (Punithalingam, 1980). *A. alternata, P. mangiferae* and *Botryodiplodia* spp. were the main pathogens associated with SER of mango under the agro-

ecological conditions of Punjab province of Pakistan (Amin *et al.*, 2011). *L. theobromae* was isolated from cv. Sindhri and established as causal organism. Inoculation by placing culture disks at one side of mango produces larger lesion as compare to other methods of inoculation.

Range of chemical fungicides is used in pre- and postharvest disease management. In present comparative studies of six fungicides (Carbendazim, Gemstar, Native, Score, Tecto and Tilt), lower used concentrations of Carbendazim followed by Tecto appeared as highly effective to inhibit the growth of *L. theobromae* on agar medium while at higher used concentration (30000 ppm) no growth of *L. theobromae* was observed with any fungicide. Khanzada *et al*. (2005) also found that Carbendazim and Thiophanate-methyl were highly effective in inhibiting the growth of the *L. theobromae.* Lower growth of pathogen occurs with increasing concentration. Nativo, Gemstar and Carbedazim at the lowest used dose (10000 ppm) reduced the lesion area while no lesion develop at 20000 and 30000 ppm in hot dip treatment at 50 ºC for 5 min. Sharma *et al*. (1994) evaluated Carbendazim, Sodium orthophenylphenate, Potassium metabisulfite, Mancozeb, Carboxin, Dodine, Iprodione and Thiabendazole were evaluated for control of *B. theobromae* on mango cv. Dashehari and found that 0.1% Carbendazim (dip treatment) was the most effective fungicide for the control of *B. theobromae* which resulted in reduction in decay indices of 90.7%. Postharvest application of Tecto (1.8 ml/L) alone and in combination with Sportak (0.5 ml/L) and Carbendazim (450mg/L) gave significantly better disease control Stem End as compared to Sportak (0.5 mL/L) alone (Amin *et al.*, 2011). Benomyl (Benlate) dip in hot water of 52ºC gives good control of stem end rot caused by both *D. dominicana* and *L. theobromae* (Coates *et al*, 1993; Johnson *et al*, 1989; Johnson *et al*., 1990). Where allowed, Carbendazim can be applied with hot water (52°C) at the recommended rate, to control of stem end rot and anthracnose (Johnson and Hofman, 2009).

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